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SOIL ACTINOMYCES AND POTATO SCAB

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The persistence of potato scab in soil infected with the pathogenic organisms inducing it is appreciated by both plant pathologists and growers, but it is not known how long a period must elapse before potatoes can be safely grown again on such soil.

Two plots of land were available for use from 1928 onwards on which accurate records of the scab percentages had been made in 1914-1917. No potatoes had been grown on one of them since 1916, corn or timothy being harvested. Susceptible potatoes on this plot had developed almost 100 percent scabby tubers in 1916 whereas a similar variety planted in 1935 showed only 26 percent of tubers slight scabbed and 3 percent with large and numerous scabs.

During eight years of the time intervening between the dates of the two trials, plate counts were made of the bacteria and actinomyces in the two soils. Soil moisture percentages, the number of actinomyces and the total numbers of organisms were determined at monthly intervals and during a part of this time the number of bacterial spores as well.

The numbers of actinomyces and their percentages of the total numbers of organisms varied widely but the actinomyces numbers in general followed the bacterial numbers and both were influenced more by the soil moisture content than by the temperature or the season of the year.

The number of actinomyces in the soil was not affected by the long crop rotation but the pathogenic species or strains producing potato-tuber scab seem either to have died out or to have changed their mode of life from a parasitic to a saprophytic one.

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By B. F. Lutman, R. J. Livingston, and Alice M. Schmidt

Introduction

The actinomyces play varied rôles in the soil, some helpful, some harmful. Thy tend to break down organic matter, thus making it available for the use of higher plants, but when present in material numbers in the soil the potato tuber disease known as corky or common scab is induced.

The growth of actinomyces mycelium into the lenticels of the small growing tubers stimulates the production of an unusually large number of irregular, thick-wall, cork cells, this tissue mass being the scab lesion. The mycelium may grow over the surface of the tuber skin and stimulate by its presence or excretions an abnormal production of cork to such an extent in some cases that the entire tuber surface may become russetted. Numerous questions regarding the persistence and activity of the organisms as well as concerning the sundry species of actinomyces remain as yet unanswered. The group is apparently a large and very diverse one, its classification still extremely uncertain and fragmentary and the life history of species in the soil more or less unknown. Problems remain unsolved with regard to potato scab itself, such as the real nature of resistance to the attacks of the organism, the number of strains or species which may produce lesions, the type of injury which they induce, whether deep or shallow, as well as the persistence of these parasitic strains in soil in which formerly they have been known to be abundant.

The problem which has been attacked in the present study is that last named: the persistence of strains of actinomyces in the soil after a long rotation of crops other than the potato, none of which harbor parasitic varieties. Three essential conditions must be met before success may be attained.

1. Access must be had to a soil or soils in which scab organisms are known to be prevalent, as shown by a high percentage of scab on potato tubers grown thereon.

2. The organisms of the scab type in such soils must be counted over a series of years.

3. After a long interval of time demonstration of scabbing by the growth of potatoes in this same soil must be made.

The practical difficulty lies in the second of these requirements since scab-producing organisms can not be readily identified by the ordinary bacteriological plating-out of the soil. Various attempts have been made

to describe a single species which will induce scab and such a species (*Actinomyces scabies*) with its morphological and physiological accounts has been admitted to plant pathology texts. If we are candid, however, we must admit that such accounts are faulty in that they are incomplete. The strain so described under suitable conditions will produce scab but so will many other very similar ones, and on the ordinary soil agar plates those which induce scab can not be differentiated from the widespread, non-pathogenic strains. However, the usual scab actinomyces produce a brown discoloration in the media. This characteristic difference may be used as a means of separating the actinomycete group into: (a) brown-color-producing colonies (the old *Actinomyces chromogenus* group); (b) non-color-producing colonies (the old *Actinomyces albus*). Such a division, although admittedly crude according to modern practice in respect to the species in this group, does serve to give one some idea of the two types.

The other two requirements are easily fulfilled, namely, a high initial percentage of infected tubers secured when a susceptible variety is grown and the percentage of infected tubers attained following a long crop rotation (omitting potatoes) when the same susceptible variety is again grown on the same soil.

The senior writer (1919) grew several varieties long years ago on certain plots, the last trial having been made in 1917. The plots were then studied in detail so that the potato-scab possibilities of their various portions were well known. No potatoes have been grown on one of them since 1917, corn and timothy hay having been the predominant crops, while the other has been almost continuously in sod save that potatoes were grown during two years on part of it. Heavy applications of garbage and pig manure were made for a number of years on one plot. The two plots were again planted in 1935 to Green Mountain potatoes, a variety that had been one of the most susceptible in previous tests.

The two soils—hereafter denoted as "farm soil" and "greenhouse soil"—are of glacial-drift origin but their textures and physical composition as well as their water-holding capacities and aeration are quite unlike, as the following table will show:

	Coarse sand	Fine sand	Very fine sand	Silt	Clay	Maximum soil moisture content in winter*
	%	%	%	%	%	%
"Farm soil"	50	48	..	..	2.	69.6
"Greenhouse soil"	..	..	27.7	64.8	7.5	77.

* Both soils frozen at the time of sampling.

The bacteriological work has been carried on under the direction and with the help of the senior writer, by Mr. A. W. Stone (1924), by the present assistant plant pathologist, Miss Winona E. Stone (1928-1931), by Mrs. Alice M. Schmidt (1931-1932) and by Mr. R. J. Livingston (1932-1935). The latter two appear as joint authors since they made many observations as to the phases in which the organisms live in the soil, the percentages of spores, spore numbers and staining of spores directly in soil smears.

PREVIOUS WORK

No attempt is made to cover the entire literature of the actinomyces, but a few papers which bear on the subject are noted. The subject, as here understood, involves the life history of actinomyces in the soil and the persistence of the parasitic strains. The general literature on the group can be found in Drechsler (1919), Krainsky (1914), Lieske (1921), Waksman (1916, 1918), Waksman and Curtis (1918), and on the *albus* group in Duche (1934).

Is a single species or are a number of species parasitic? A single species, *Actinomyces scabies,* as the sole activator of the cork cambium has been accepted by many plants pathologists although Lutman and Cunningham (1914) pointed out that a number of strains of the old group-species, *A. chromogenus,* could induce scabbing. More recent work on the multiple origin of scabs or on variations in infection has been done by Millard and Burr (1926), Sanford (1926), Millard and Taylor (1927), Wollenweber (1923), Schlumberger (1929), Berkner (1933) and Dippenaar (1933). Attention should be called to the literature on the presence of parasitic strains in virgin soils (Lutman, 1923) and to the persistence of such strains in soil for many years after its use for potato culture had been abandoned. The above references likewise cover this phase. The conditions of infection are discussed therein, as is also the relation of the soil actinomyces to other soil bacteria. Certain soil factors favor the multiplication of the ordinary soil bacteria while others induce an increase in the actinomyces. The numerical changes may be the consequence of these sets of factors or they may result from an actual antagonism between the two groups, an antagonism based on their byproducts.

SEASONAL RELATIONS.—The soil bacterial populations at various seasons of the year have been studied. Unexpectedly high numbers have been found to be present during the winter months and considerable theorizing has been done as a result. Conn (1910, 1912) found unlike summer and winter floras, the latter being apparently unrelated to the soil moisture content, while the former was thus related. Brown and Smith (1912) agree in general with Conn's statements to the effect that winter counts were higher than those made in the summer and suggest that the hygroscopic moisture held around the soil grains does not freeze although the soil itself is frozen and that the bacteria increase in numbers in this hygroscopic layer. Conn (1914), having done further work, holds that the freezing of the soil water breaks up the clumps of bacteria in such a complete manner that the individuals separate in the shaking of the sample in the dilution flasks and appear as colonies on the plates. He also supports the hygroscopic water theory suggested by Brown and Smith. Waksman (1916), plating out soil from New Jersey where freezing only occurs for intervals of 8 to 10 days, found high counts in frozen soil, but not the highest for the year. Harder (1916) assumes that the apparent soil bacterial increases after a heavy frost are due to an upward movement of soil water with a resultant increase in the water content of the surface layers, the latter being the dominant factor in soil counts. He modified this

assumption a little later (1918) and placed the emphasis on extra- or intra-cellular enzymes. Vass (1919) found no actual increase in bacterial numbers, but a breaking-up from freezing of the bacterial clumps. Waksman (1916) did not accept the contention as to the correlation between high soil water content and bacterial soil content. Cutler *et al.* (1922) offer no explanation for variations in soil moisture, rainfall, soil temperature, or seasonal temperature, but do find two maxima for bacterial counts, one at the end of June, the other at the end of November. Lochhead (1926) supported the summer and winter micro-flora hypothesis. Berry and Magoon (1924), working on frozen food rather than on soil, suggest that "the physical condition of the refrigerated material and the environmental factors seem to be more important than the degree of cold in determining whether or not growth will take place"; that "the increase in the numbers of organisms in frozen soils, attributed by several workers to the development of organisms especially adaptable to low-temperature conditions, might be due, it would seem, to changes in food material, the formation of ice resulting, perhaps, in a more favorable concentration of the nutrients or in alteration in its composition."

NUMBERS.—Beijerinck (1900) found actinomyces to a depth of two meters and that they were relatively more abundant at that depth than other soil bacteria. Hiltner and Störmer (1903) found them to form 20 percent of the total bacterial count in the spring and 30 percent in the fall. Fousek (1912) also perceived that higher percentages existed in the fall than in the spring and pointed out that while they formed from 20 to 30 percent of the numbers in loam soils, they only showed from 8 to 15 percent in clay and from 7 to 10 percent in sand, and that fallow contained more than cultivated soils. Krainsky (1914) found 30 percent of the total organisms of a soil sample plated on calcium malate to be actinomyces. Waksman and Curtis (1918) found the following numbers of actinomyces to the total numbers of organisms at 1 inch, 743,000 to 7,340,000; at 4 inches, 933,000 to 5,300,000; at 8 inches, 612,000 to 2,710,000; at 12 inches, 239,000 to 950,000; at 20 inches, 246,000 to 259,000; at 30 inches, 240,000 to 124,000. Conn (1916-b) noted an average of 37.5 percent of the total flora of sod soil to be actinomyces. Since they occur on roots of plants and most frequently in sod soil, he later (1917) intimated that they might be an active agent in the decomposition of grass roots a suggestion which seemed to be confirmed by a study of a sample of soil divided into portions, one containing no grass roots and the other filled with them. The number of actinomyces remained about the same in the untreated plot while they increased to 6,000,000 in the one containing grass roots and remained constant in number for 10 months.

Waksman and Curtis (1918) obtained percentages varying from 3.5 to 46 in 25 soil samples from various parts of North America and from the Hawaiian Islands. Conn (1918) observed more conidia than bits of mycelium in the soil. Snow (1926) states that 52.4 percent of the colonies derived from a wind blown, desert soil were actinomyces. Waksman (1926) found few soil species of actinomyces capable of destroying cellulose but, on the contrary, Waksman and Dubos (1927) held that they were the leading cellulose destroyers in dry soils and that they are not exterminated as are the other soil bacteria by the soil amœbæ. Jensen (1930) found the numbers in Danish soils to vary from a few thousand to 13,000,000 per gram, the very acid (pH 5 or —) soils containing few while the high counts were most frequent on soils with a pH 6 to 8. He feels that soil reaction is the main factor affecting the numbers. Morrow (1931) obtained high actinomyces percentages in mesophytic (medium-dry land) associations while the bacterial percentages were high and actinomyces percentages low in marsh (hydrophytic) associations.

Smith and Humfeld (1930) on both sand and clay loams, limed and unlimed, found the number of actinomyces diminished when the other bacteria were most abundant after the turning under of green manures.

Itano and Arakawa (1932) believed the ammonification of rape-cake to be due to actinomyces rather than to bacteria. Frutchey and Muncie (1934) correlated actinomyces and soil moisture percentages as follows: Moisture: 20, 35, 55, 70, 100 percents; actinomyces: 8.1, 12.3, 10.6, 6.4, 8.5 percents. The relative actinomyces numbers increased when soil moisture percents were low, whereas lower counts were obtained when high moisture percentages were found in the presence of the various mercurial disinfectants which they were using on the soil.

CONDITION OF SOIL ORGANISMS.—Few data seem to have been accumulated on this phase of bacterial life. Conn (1918) was of the opinion that the percentage of spores of the total soil flora was small, amounting probably only to about one percent, his conclusions being based on his direct-count method. Joffe and Conn (1923) found high moisture content and organic nitrogen favorable to vegetative growth. They feel that "there is reason to believe that the rôle of spore-forming bacteria in soil is, under ordinary conditions, one of watchful waiting. They seize upon bits of organic matter and utilize periods of high moisture content to grow occasionally for a few generations before going back to the spore state, and thus maintain their numbers." On dry, wind-blown soils, Snow (1926) found 42.6 percent of the organisms were spore bearers, but the number present in the soil as spores was apparently not determined. The actinomyces were described by Conn as present in the soil both as bits of mycelium and as spores, the latter predominating.

The processes of staining bacteria according to the direct-count method have been discussed in the paper already noted. The differentiation of the spores in soil samples on which the direct-count method is used is generally recognized to be a very difficult process since they are so similar in their staining reactions to the vegetative bacteria. The usual carbol fuchsin-methylene blue procedure gave these investigators no differentiating results on soil spores. The spore stain of Schaeffer and Fulton (1933), based on a suggestion by Wirtz (1908), and the long and intensive drying of the slides with their adherent material suggested by Lote (1931), were tried with only indifferent success by the writers (p. 25).

RESISTANCE OF BACTERIAL SPORES TO HEAT.—The resistance of bacterial spores to heat and drying has been ascribed by most bacteriologists to a combination of a very greatly desiccated protoplasm surrounded by a very thick wall impregnated with some fat-like substance which renders it quite impermeable. However, this assumption is not uniformly accepted. Thus Virtanen (1934) has suggested that enzymes are responsible for spore germination and that spore death by heat is due to a destruction of the germination enzymes and not to a heat coagulation of the cell proteins.

Resistance of soil bacteria to death by heating, even through no spores can be observed in the sample by staining methods, may be due to the very high osmotic pressure of the soil solution, especially when in contact with the soil particles. The spore walls and concentrated protoplasm of spores in culture tubes may not be necessary to preserve them from destruction by heat. Fay (1934) confirmed, on *Escherichia coli*, the work of Robertson (1927) on thermoduric bacteria indicating that hypertonic sugar solutions protected ordinary non-spore bearing bacteria from death heat.

The thermal death point of the actinomyces is difficult to determine with accuracy. Their spores are only bits of mycelium which have broken off and serve the purpose of reproduction. The two physical properties which are considered characteristic of bacterial spores are lacking, namely, a dense, highly concentrated protoplasm and thick, impermeable walls.

Before discussing the thermal death point of the actinomyces, the fact should be noted that this word phrase "thermal death point" has a quite definite concatena-

tion in bacteriology as applied to the ordinary bacteria in a vegetative condition, implying the temperature at which organisms are killed when exposed for 10 minutes either in distilled water or in a liquid medium. The only variable factor, therefore, is temperature. Esty (1928) has pointed out in this connection that "there is no time and temperature combination which alone may be defined as the thermal death point, as the time varies with the temperature at which the organisms are heated. There is, however, a definite time-temperature relationship, the time decreasing as the temperature increases. In view of this the term "thermal death tissue" probably more appropriately designates this relationship than "thermal death point."

Lieske (1921) found that actinomyces spores withstand freezing temperatures of 8° to 10° C. for long periods of time without apparent injury. On the other hand, he observes that all actinomyces are sensitive to high temperatures, most species being killed by a short heating at 60° C. The conidia (spores), born aerially, are more resistant than the vegetative threads, but a short boiling destroys them, indicating that they do not have the same high resistance as do the spores of most bacteria.

Sanford (1926) killed the aerial spores of *Actinomyces scabies* in sterile water by a temperature above 90° C. for 10 minutes, whereas they germinated after 20 minutes' exposure at 83° C., although 20 minutes at 85° killed them all.

Waksman (1927) states that "all actinomyces are very resistant to drying, as indicated by the fact that they are found abundantly on dry straw, hay, and soil. Direct sunlight does not exert any injurious effect upon the actinomyces and does not modify their growth. Exposure to ultra-violet rays for 10 minutes does not affect them; after one hour the organism is definitely affected but not destroyed." He further states that the thermal death point of most actinomyces is 75° C. After 20 minutes' exposure the mycelium may survive at 60° C., but is killed at 70° C.; the spores may survive for 20 minutes at 75° C., but are killed in 30 minutes.

METHODS

Two methods are available for the determination of the numbers of soil bacteria; the usual plating out procedure with its various modifications and the direct staining method on a glass slide, also with many modifications. Comments will be made later (pp. 24-26) on the plating method and the taking of samples but no detailed discussion is necessary as the usual procedures were followed.

The direct-count method, which was introduced by Conn (1917-b) and which has been considerably modified (Conn, 1918, 1928; Conn and Thatcher, 1927) is very useful although it has its inaccuracies as its authors fully recognize. Further modifications have been suggested by Winogradsky (1924, 1925). The growth of soil organisms on a slide imbedded in the soil, the clumps which have developed being subsequently stained, was suggested by Rossi (1928) and improvements in procedure have been made by Cholodny (1930), Conn (1932), and Cholodny (1934).

Soils.—Two unlike soils were used for the resistance test in 1914-1917 and for bacteriological studies since that time. That designated as

"Farm" is a very light sandy loam containing considerable humus (pH 6.8); that termed "Greenhouse"—not because the soil was contained in or the work done in a greenhouse but because it was located near the University greenhouse—is a very heavy, fine-grained clay (pH 6.8). Both soils are of glacial drift origin.

Plating out methods.—The methods employed have been somewhat modified during the years. At first a nutrient agar was used to which one percent dextrose had been added; when the work was resumed in 1928, plain agar was employed; then, later, Bacto-nutrient agar, a medium which contained no sugar. The latter has been used continuously during recent years.

Accuracy of sampling is obviously important. All samples were taken at a depth of above three inches, the surface soil having been scraped away. For some years they were taken from four holes at this depth and each placed in a clean glass jar; but during 1935, in order to get a composite picture of the microbial flora, they were taken from the margin of a single larger hole and similarly placed in a jar. Each sample was passed through a fine-meshed sieve in order to remove small stones and plant débris and from it, thus mixed, one gram was weighed into a 100 c.c. dilution flask. After thorough shaking, further dilutions of 1 to 10,000 and 1 to 1,000,000 were made. One c.c. of this last dilution was placed in each of 20 dishes and the melted agar added. After incubation at room temperature (16° to 30° C.) for 10 days, the counts showed from 10 to 20 bacterial colonies per plate. The actinomyces colonies could be recognized in most cases but were always examined with the low power of the compound microscope. They were roughly divided into the pigment producers (*A. chromogenus* group) and the white or non-color producers (*A. albus* group). This procedure was modified during 1934 and 1935, four one-gram samples instead of one being removed from the sifted soil and diluted as before indicated. Ten plates were then made from each of these one-gram samples and the counts averaged.

Soil moisture contents varied widely at different seasons during the year, so in order to determine the facts 100 grams of each composite sample was air dried for several days and then oven dried for two to three hours at 100° C. Organism numbers are computed on the basis of one gram of dry soil.

Direct microscopic count methods.—The original method used for counting the organisms directly was that developed by Conn (1918). One gram of soil was mixed and thoroughly shaken with 10 c.c. of a 0.015 percent sterile gelatin solution, and allowed to stand for five min-

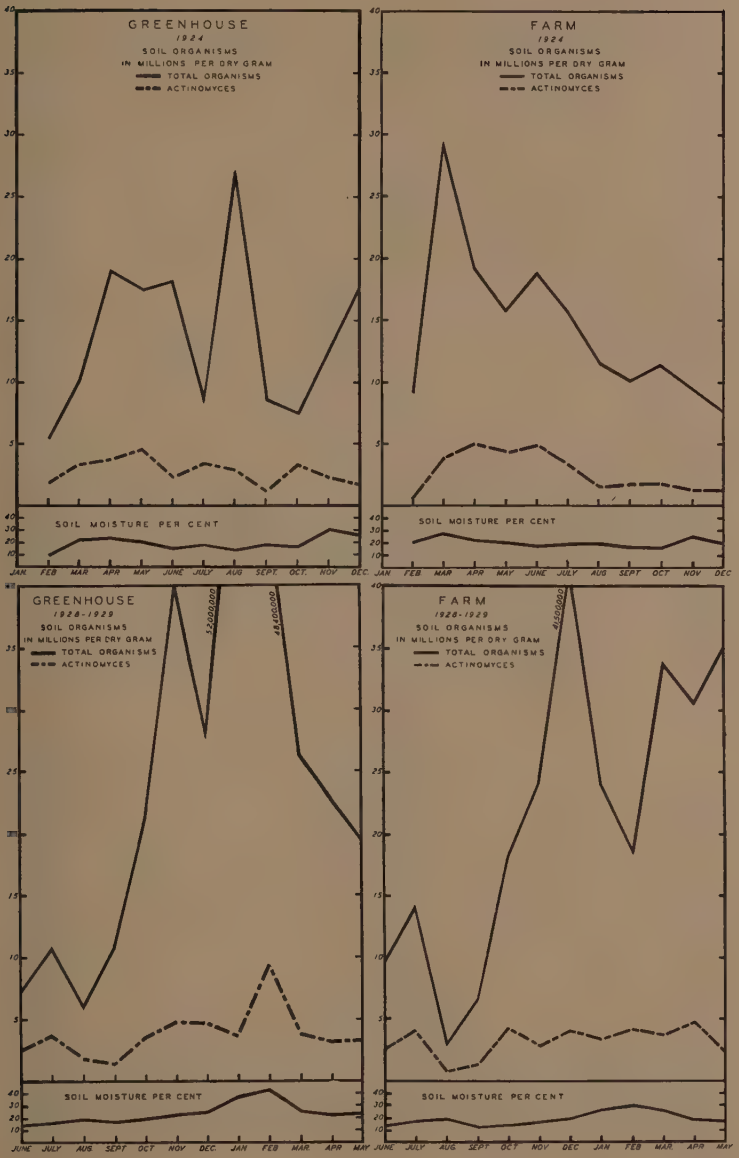
utes, during which time the heavier and larger soil particles fell to the bottom leaving the bacteria suspended in the supernatent liquid, from which 0.01 c.c. was drawn with a capillary pipette and spread over one square centimeter on a glass slide. Fourteen slides were thus prepared, then dried with moderate heat and stained with a heated three-percent aqueous solution of erythrosin or with a one-percent aqueous solution of rose bengal. They were then examined with a 2-mm. apochromatic objective combined with a 15x compensating ocular. Fifty fields were counted on each smear, especial attention being given to the actinomyces, 0.25 to 8 of which appeared in a field. Only actinomyces which showed as threads were counted, the spores being impossible to distinguish as such.

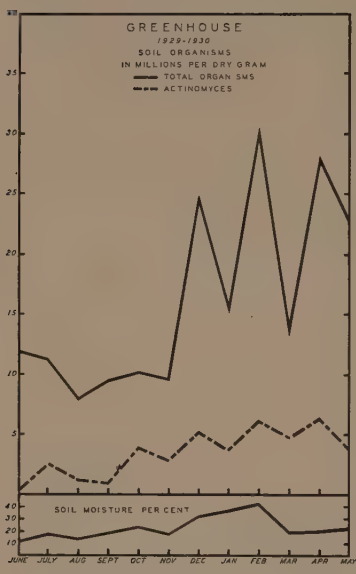
Plates were poured of the same samples (with the 1-1,000,000 dilution) in order to compare the numbers, especially of actinomyces, obtained by direct count and by plating-out.

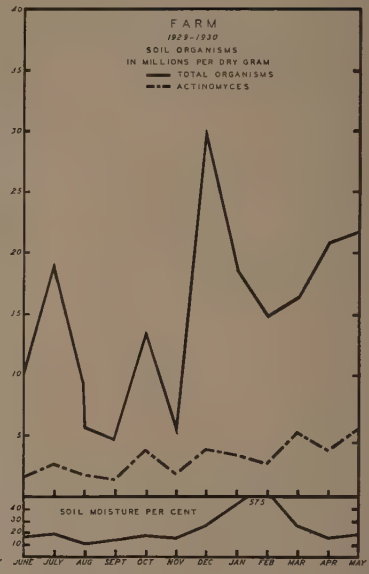
Spore counts on plates from soil samples.—Two types of spores were sought for counting, bacterial and actinomycelial. In order to make certain that the latter might be seen the death temperature for the vegetative forms of the bacteria had to be carefully regulated. The plates were made as usual from the 1-1,000,000 dilution. Four flasks containing 1-10,000 dilutions were then heated in a water-bath for 15 minutes at 75-85° C. in order to kill the actinomyces mycelium and, if possible, to avoid injuring the spores.[1] Immediate cooling followed. From each flask 10 one-c.c. portions were placed in 10 petri dishes, a total of 40 plates, and agar added. Usually, from 50 to 150 colonies grew on these heated-solution plates.

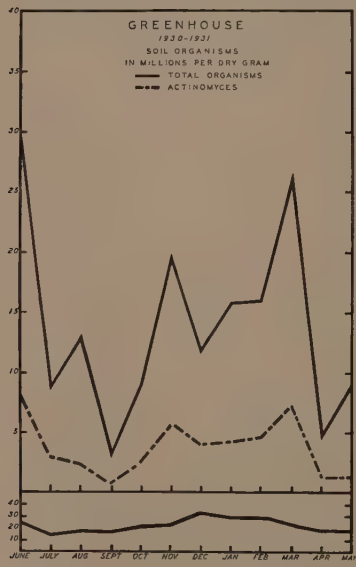
Staining spores directly in soil smears.—Conn (1918, 1928) claimed to be able, without using a special stain, to differentiate spores from vegetative bacteria by their staining reactions and shapes, the latter taking a stain more thoroughly than the former. The writers, being unable to determine spores with accuracy, resorted to the standard spore stain, carbol-fuchsin decolorized from vegetative cells by acid-alcohol and counterstained with methylene blue, giving red spores in blue bacteria. Repeated trials were unsuccessful in producing differentiation. Satisfactory preparations from bacterial cultures were obtained by the use of Schaeffer and Fulton's (1933) stain consisting of malachite green and safranin, which gave green endospores and red bacteria. This stain, as will be indicated later (p. 25), afforded some results on soil smears but they were not always consistent.

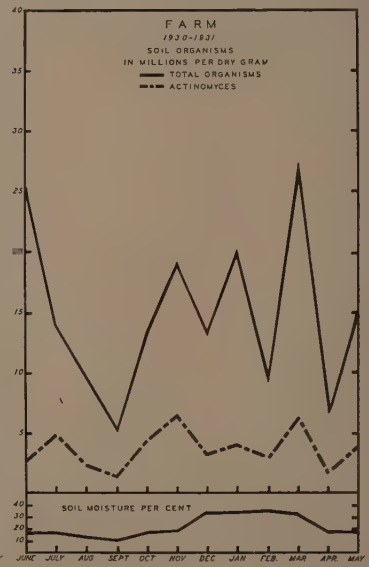
[1] 80°-85° was first used but, fearing destruction of actinomyces spores, 75°-80° was used in later trials.

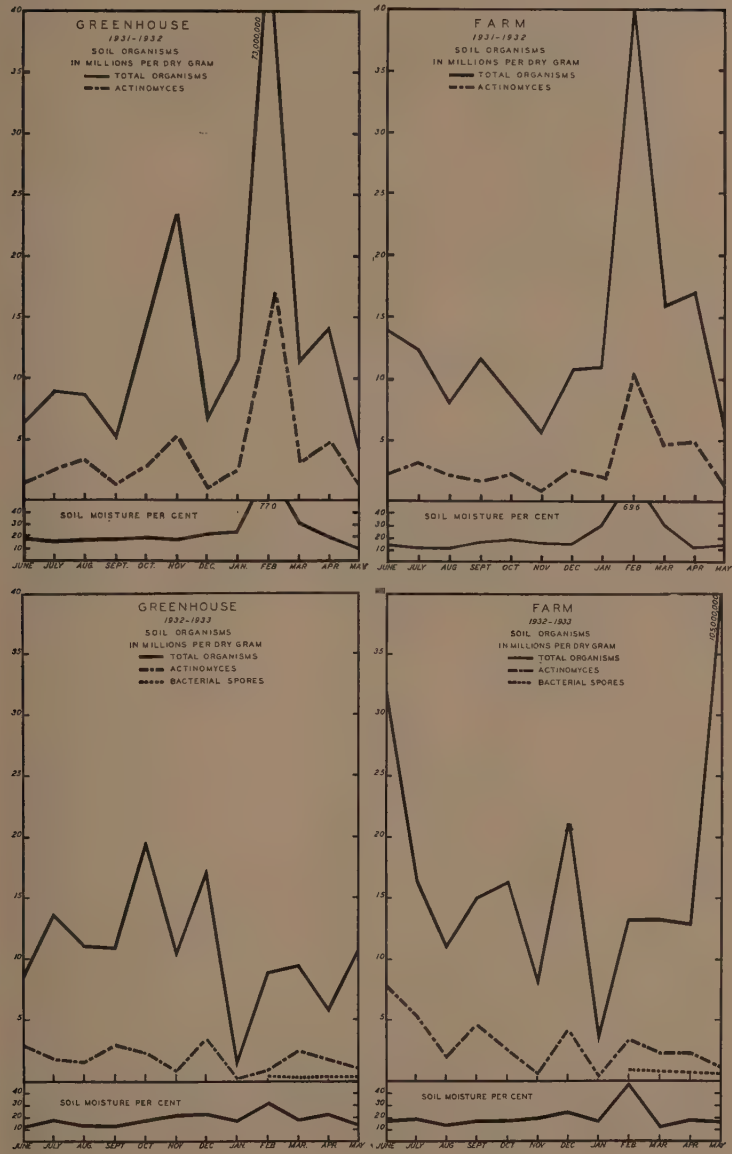

GREENHOUSE
1924
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
SOIL MOISTURE PER CENT
JAN FEB MAR APR MAY JUNE JULY AUG SEPT OCT NOV DEC
FARM
1924
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
SOIL MOISTURE PER CENT
JAN FEB MAR APR MAY JUNE JULY AUG SEPT OCT NOV DEC
GREENHOUSE
1928-1929
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
32000000
48400000
SOIL MOISTURE PER CENT
JUNE JULY AUG SEPT OCT NOV DEC JAN FEB MAR APR MAY
FARM
1928-1929
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
48400000
SOIL MOISTURE PER CENT
JUNE JULY AUG SEPT OCT NOV DEC JAN FEB MAR APR MAY

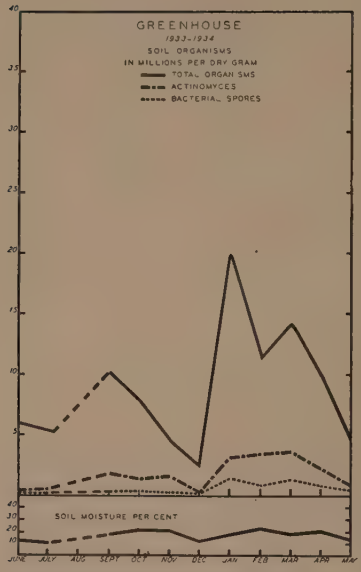

GREENHOUSE
1929-1930
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
SOIL MOISTURE PER CENT
JUNE JULY AUG SEPT OCT NOV DEC JAN FEB MAR APR MAY

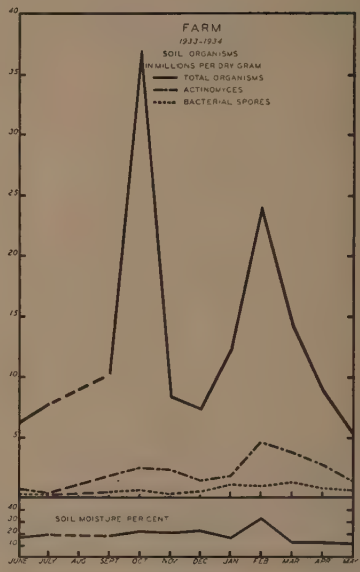

FARM
1929-1930
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
SOIL MOISTURE PER CENT
575
JUNE JULY AUG SEPT OCT NOV DEC JAN FEB MAR APR MAY

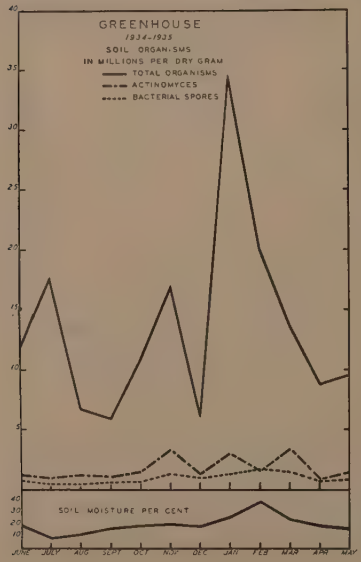

GREENHOUSE
1930-1931
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
JUNE JULY AUG SEPT OCT NOV DEC JAN FEB MAR APR MAY

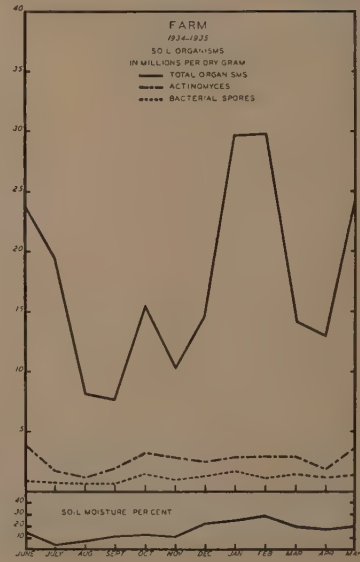

FARM
1930-1931
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
SOIL MOISTURE PER CENT
JUNE JULY AUG SEPT OCT NOV DEC JAN FEB MAR APR MAY

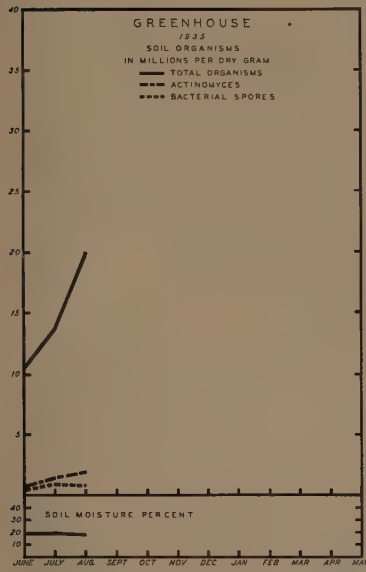

GREENHOUSE
1931-1932
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
23,000,000
SOIL MOISTURE PER CENT
770
JUNE JULY AUG. SEPT. OCT. NOV DEC. JAN. FEB MAR APR MAY
FARM
1931-1932
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
SOIL MOISTURE PER CENT
696
JUNE JULY AUG SEPT OCT NOV DEC JAN FEB MAR APR MAY
GREENHOUSE
1932-1933
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
BACTERIAL SPORES
SOIL MOISTURE PER CENT
JUNE JULY AUG. SEPT OCT NOV DEC JAN FEB MAR. APR MAY
FARM
1932-1933
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
BACTERIAL SPORES
43,000,000
SOIL MOISTURE PER CENT
JUNE JULY AUG SEPT OCT NOV DEC JAN FEB MAR APR MAY

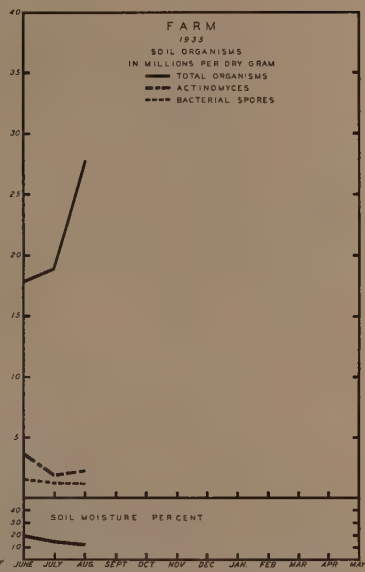

GREENHOUSE
1933-1934
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
BACTERIAL SPORES
SOIL MOISTURE PER CENT
JUNE JULY AUG SEPT OCT NOV DEC JAN FEB MAR APR MAY

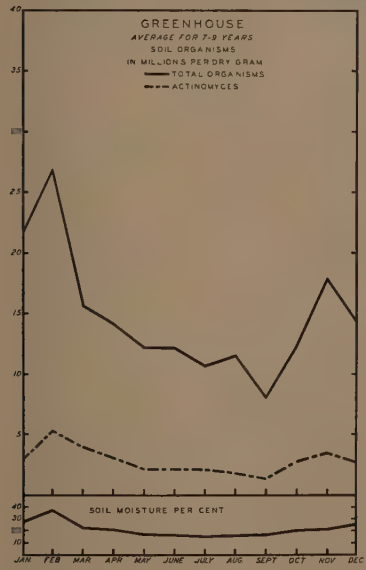

FARM
1933-1934
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
BACTERIAL SPORES
SOIL MOISTURE PER CENT
JUNE JULY AUG SEPT OCT NOV DEC JAN FEB MAR APR MAY

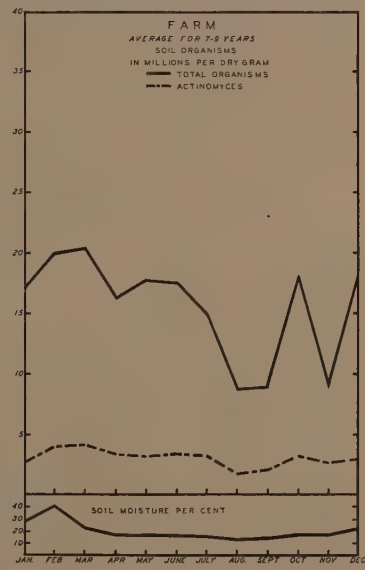

GREENHOUSE
1934-1935
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
BACTERIAL SPORES
SOIL MOISTURE PER CENT
JUNE JULY AUG SEPT OCT NOV DEC JAN FEB MAR APR MAY


FARM
1934-1935
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
BACTERIAL SPORES
SOIL MOISTURE PER CENT
JUNE JULY AUG SEPT OCT NOV DEC JAN FEB MAR APR MAY


GREENHOUSE
1935
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
BACTERIAL SPORES
SOIL MOISTURE PERCENT
JUNE JULY AUG. SEPT OCT NOV DEC JAN FEB MAR APR MAY


FARM
1935
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
BACTERIAL SPORES
SOIL MOISTURE PERCENT
JUNE JULY AUG. SEPT OCT NOV DEC JAN. FEB MAR APR MAY


GREENHOUSE
AVERAGE FOR 7-9 YEARS
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
SOIL MOISTURE PER CENT
JAN. FEB MAR APR MAY JUNE JULY AUG. SEPT OCT NOV DEC


FARM
AVERAGE FOR 7-9 YEARS
SOIL ORGANISMS
IN MILLIONS PER DRY GRAM
TOTAL ORGANISMS
ACTINOMYCES
SOIL MOISTURE PER CENT
JAN. FEB MAR APR MAY JUNE JULY AUG. SEPT OCT NOV DEC

RESULTS

PLATE COUNTS OF THE TWO SOILS CORRELATED WITH THEIR MOISTURE CONTENTS

The results thus obtained are displayed as graphs (pp. 11-15). Actual bacterial counts are used rather than their logarithms. Several points should be noted. Owing to the conditions under which the work was done, the "years" do not always begin or end on the same month. Furthermore some of the later graphs show spore counts. All graphs show total numbers of organisms (including actinomyces), the number of actinomyces and the soil moisture percentages.

Composite graphs of the data for three factors, namely, averages of total organisms, of actinomyces and of soil moisture percentages follow the year-by-year graphs on page 15. By taking a period as long as the one shown—eight years—it was thought that marked seasonal diversities, if any, would be so counterbalanced that safer conclusions might be drawn therefrom.

The results secured during the last three years (part of 1933, all of 1934, and part of 1935) on soil moisture percentages, the total numbers of organisms, the numbers which resist heating at 80°-85° C. (or 75° C.-85° C.), *i.e.*, the number of spores, are shown in the accompanying graphs (pp. 13-15).

RELATIONS OF BACTERIAL NUMBERS, SEASONS, SOIL MOISTURE PERCENTAGES AND ACTINOMYCES PERCENTAGES

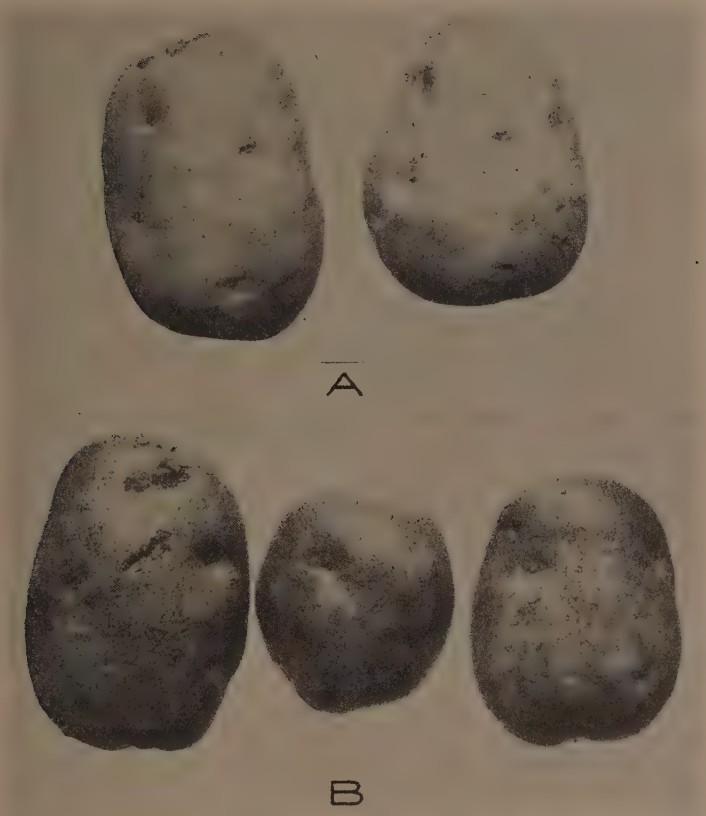
Bacterial numbers.—The most striking feature is their extreme variability.

1924—Two peaks on farm soil, March and August; two peaks on greenhouse soil, April and June.
1928-1929—Peak for both soils, October to February.
1929-1930—No pronounced peak but organisms more abundant November to April.
1930-1931—Starting with a high count in June; another rise in October to March.
1931-1932—A peak on both soils during January to March.
1932-1933—Two peaks, June to July and May on farm and October to December on greenhouse soil.
1933-1934—On farm soil, a peak in October and again in February to March; on greenhouse soil, a winter peak of January to April.
1934-1935—A winter peak, December to March on both soils.

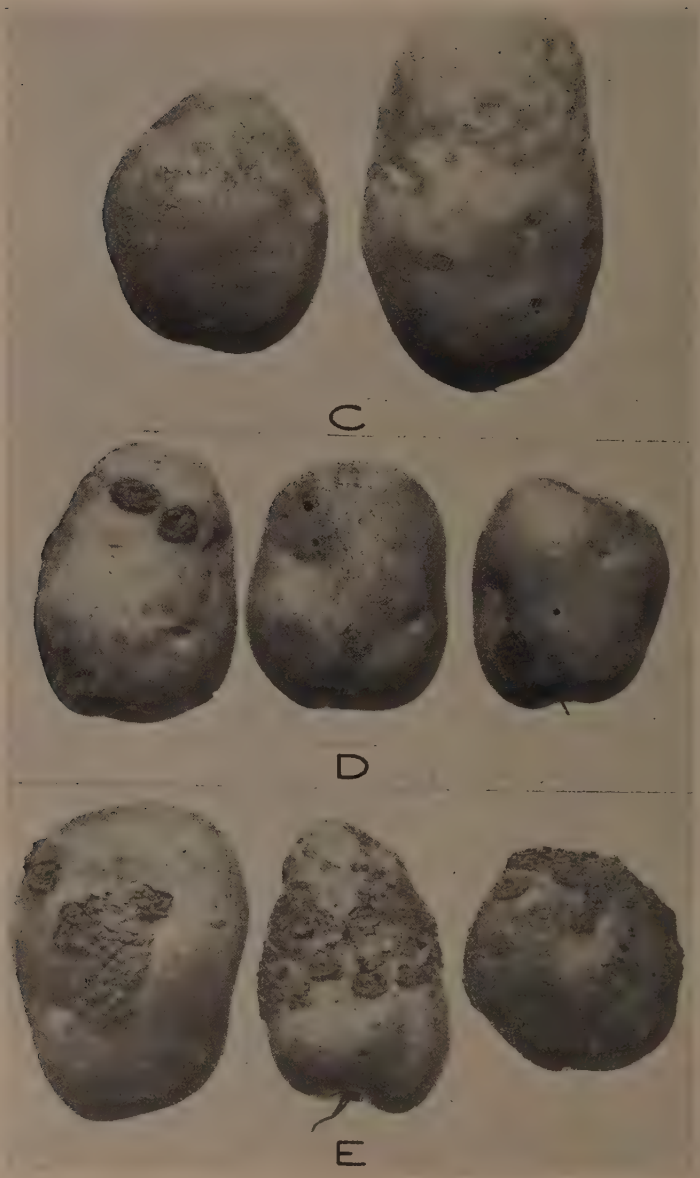
In general, rise in numbers seems likely to occur in the fall and to continue through the winter, but this does not hold in all cases. In fact in some years, such as 1932-1933, the exact reverse situation arose.

PLATE I.—Fig. 1. Colonies of: A. *Actinomyces albus;* B. *A. chromogenus.* Fig. 2. Margin of an actinomyces colony. Fig. 3. Spores of an actinomyces. Fig. 4. Mycelium of an actinomyces. All figures are from pure cultures.

PLATE II.—Fig. 5 and Fig. 6. Actinomyces mycelium as it appears in soil smears. M. No spores could be found or identified as such.



PLATES III AND IV.—Photographs showing the various gradations of clean and scabby tubers taken from the plot. A, B, and C were counted as clean although distinct russetting is shown on B and C. D was considered to be slightly scabby and E was badly scabby.


C
D
E

Bacterial numbers and soil moisture percentages.—The relation of the soil moisture content to the total number of organisms is just as confusing and erratic, as the following observations indicate.

1924—A peak in soil moisture percentage followed but did not precede the rise in numbers of total organisms in the fall.

1928-1929—The rise in soil moisture was followed by a sharp rise in the total numbers of organisms.

1929-1930—A similar rise in soil water content and bacterial numbers occurred during the winter months.

1930-1931—A sharp rise in soil water content occurred during the winter months as well as marked bacterial increase, although the minimum water content accompanied the low bacterial count in September.

1931-1932—As in the preceding year low bacterial counts in June to October are accompanied by low soil-moisture percentages, both increasing in the winter season.

1932-1933—The two soils must be considered separately. The soil moisture content on the farm soil did not fluctuate as widely as in some years. The extremely high bacterial content of May was due to a heavy top dressing of pig manure made a few weeks before the samples were taken. The moisture content and the bacterial count data on the greenhouse soil were irregular, but the changes in the latter tended to follow those in the former.

1933-1934—The farm soil bacteria were again influenced by an application of pig manure in August which sent the counts up and kept them up until November. The winter rise in counts was accompanied by a small rise in water content. The greenhouse soil bacteria were more numerous during the winter months than at other times, although the water percentages held fairly constant.

1934-1935—On both soils, low summer bacterial counts accompanied low soil moisture percentages while high winter bacterial counts paralleled high soil moisture percentages

The bacteria found in the soil and which produce colonies on nutrient agar plates may have little significance since many if not most of them are denitrifers and general saprophytes. Since emphasis in this paper is laid on the actinomyces, especially those of the potato scab-producing group, an examination of the data on these counts may be useful. Comparisons will be made between total numbers of organisms and soil, water contents and between total numbers of actinomyces and the total of all microorganisms with special reference to seasonal variations and to soil water content fluctuations.

ACTINOMYCES TOTAL NUMBERS, PERCENTAGES, RELATION TO SOIL WATER CONTENTS

1924—Actual numbers were highest during the summer months and dropped off in the fall. Percentages, however, remained constant in the two soils. The soil moisture was normally low during the mid-summer months.

1928-1929—The counts were about the same throughout the year. Percentages were highest in the summer and dropped off during the winter, whereas the soil moisture content was low during the summer and high in the winter.

1929-1930—The preceding years results were reversed so far as actinomyces were concerned, they being lower in numbers in summer than in winter. The percentages were irregularly the same as the numbers. The soil moisture content was high when the numbers of actinomyces were high, i.e., in the winter.

1930-1931—A small drop in total numbers during the summer and September was followed by a rise during the winter. The percentage relation of actinomyces to total organisms was irregular. The soil moisture content was high during the winter months but neither actinomyces line followed the moisture line.

1931-1932—The numbers were low during the summer but high during the winter. On the other hand, the percentages were high during the summer and low during the winter. The high count (but low percentage) of actinomyces was coincident with the winter high soil moisture content.

1932-1933—No marked seasonal changes occurred in numbers nor in percentages. The high soil moisture content was without effect during the winter months.

1933-1934—The actinomyces were high from January to April as were the other organisms. Percentages remained constant. The late winter, high soil moisture content produced highs in the counts of total organisms and of actinomyces.

1934-1935—Low summer counts of actinomyces were followed by higher ones in the fall and winter. The percentages again remained constant. The soil moisture rose during the winter months as did the total numbers of organisms and actinomyces.

SPORES; TOTAL NUMBERS; PERCENTAGES OF TOTAL NUMBERS

Between February, 1933 and August, 1935, the number of spores in the soil was determined in the manner heretofore described (p. 10). The procedure used gives the total numbers. Previous data are scarce on this point, although numerous counts are available on the number of spore formers in the soil. The latter may include two very different phases; the spores actually present in the soil and the vegetative bacteria capable of producing spores. The latter are much more numerous than the spores themselves as the data on pages 13 to 15 show.

Comparisons of the soil moisture contents, of the total numbers of organisms, the numbers which appeared on plants after heating the 1-10,000 dilution to temperatures 80°-85° C., and the percentage of spores are shown in the tables 1 and 2.

1933—The number of spores was higher in the farm than in the greenhouse soil.

1933-1934—The number of spores was generally higher in the farm than in the greenhouse soil.

1934-1935—The differences in spore content of the two soils were not very marked, but the numbers were higher in the farm than in the greenhouse soil.

These results were to be expected since a light sandy loam tends to favor the spore phase, if the organisms are spore-formers.

DATE	TYPE OF SOIL	TEMPERATURE USED	PERCENT MOISTURE IN THE SOIL	NUMBER OF ORGANISMS PER DRY GRAM		PERCENT SPORES
				IN UNHEATED SOIL INFUSION	IN HEATED SOIL INFUSION	
1933 FEB	GREENHOUSE	80-85°C	30.9	8,900000	267,000	3.0
MAR.			18 5	9,426,000	145,000	15
APR.			22.3	5,741,000	217,000	38
MAY			14.8	10,700,000	180,000	1.7
JUNE			12.8	5,877,000	144,000	2.5
JULY			9.1	5,100,000	192,000	3.8
AUG.				NO COUNTS	NO COUNTS	
SEPT.			17.6	10,012,000	187,000	1.9
OCT.			20.5	7,737,000	321,000	4.1
NOV.			20.0	4,406,000	156,000	3.5
DEC			11.7	2,291,000	70,000	3.1
1934 JAN		75-85°C	17.7	19,584,000	1,371,000	7.0
FEB.			23 7	11,239,000	714,000	6.3
MAR.			18.2	14,028,000	1,216,000	8.7
APR.			19.1	9611,000	695,000	7.2
MAY			13.2	4,205,000	366,000	8.7
JUNE			19.2	11,757,000	620,000	5.3
JULY			8.9	17536,000	338,000	1.9
AUG.			12.4	6,650,000	361,000	5.4
SEPT.			17.9	5,877,000	477,000	8.1
OCT.			19.3	10,874,000	570,000	5.2
NOV.			20.8	16,848,000	1,187,000	7.0
DEC.			18.3	6,120,000	848,000	13.9
1935 JAN			27.5	34,310,000	1,137,000	3.3
FEB.			39.6	19,909,000	1,667,000	8 3
MAR.			24.5	13,510,000	1,236,000	9.2
APR.			19.5	9,286,000	508,000	5.5
MAY			16.8	9,495,000	736,000	7.8
JUNE			18.5	10,644,000	469,000	4.4
JULY			18.8	13,762,000	916,000	6.7
AUG.			19.0	20000,000	827,000	4 1

TABLE 1.—Moisture, organisms, and spores in greenhouse soil, February, 1933 to August, 1935.

Considerable difficulty was encountered in maintaining a constant temperature as high as 80-85° C. around the flask containing the dilutions. The currents which were set in motion by the heat applied beneath the vessel containing the flask rendered equal heating throughout the body of water impossible. In the later trials, therefore, the dilutions were placed in small vials and these were immersed in water in a DeKotinsky constant-temperature apparatus, heated by electricity, and the water stirred by a small electric turbine. With the latter outfit, a temperature control within one degree C. was possible in all parts of the water surrounding the vials.

DATE	TYPE OF SOIL	TEMPERATURE USED	PERCENT MOISTURE IN THE SOIL	NUMBER OF ORGANISMS PER DRY GRAM		PERCENT SPORES
				IN UNHEATED SOIL INFUSION	IN HEATED SOIL INFUSION	
1933 FEB	FARM	80-85°C	45.6	13,051,000	748,000	5.7
MAR			11.0	13,095,000	650,000	5.0
APR.			17.8	12,835,000	558,000	4.4
MAY			15.5	105,231,000	497,000	0.5
JUNE			15.4	6,235,000	244,000	3.9
JULY			18.9	7,800,000	134,000	1.7
AUG				NO COUNTS	NO COUNTS	
SEPT			13.8	10,210,000	455,000	4.4
OCT			21.1	36,650,000	549,000	1.5
NOV			20.0	8,433,000	235,000	2.8
DEC			22.8	7,449,000	471,000	6.3
1934 JAN		75-85°C.	15.5	12,308,000	1,000,000	8.1
FEB			34.4	23,932,000	962,000	4.0
MAR			12.9	14,351,000	1,193,000	8.3
APR			12.1	8,845,000	714,000	8.1
MAY			11.5	5,305,000	521,000	9.8
JUNE			14.9	23,766,000	873,000	3.7
JULY			4.3	19,410,000	650,000	3.3
AUG			7.4	8,120,000	609,000	7.5
SEPT			11.8	7,511,000	610,000	8.1
OCT.			14.2	15,239,000	1,432,000	9.4
NOV			11.0	10,206,000	946,000	9.3
DEC			22.4	14,536,000	1,192,000	8.2
1935 JAN			24.5	29,536,000	1,588,000	5.4
FEB			28.0	29,757,000	1,000,000	3.4
MAR			19.0	14,136,000	1,379,000	9.8
APR.			16.3	12,903,000	1,011,000	7.8
MAY			19.0	24,074,000	1,277,000	5.3
JUNE			19.7	17,870,000	1,511,000	8.5
JULY			14.8	18,900,000	1,140,000	6.0
AUG.			13.0	27,600,000	1,140,000	4.0

TABLE 2.—Moisture, organisms, and spores in farm soil, February, 1933 to August, 1935.

The effects of the lowering of the temperatures for killing the spores from 80-85° C. to 75-85° C. is apparent in the increase in the percentages of spores surviving this latter treatment. While a few of the spore percentages were lower after the modified temperature, in general they were markedly higher and probably represented more nearly the organisms that were in a spore stage. With the constant-temperature apparatus, 80° C. could be regularly maintained. The spore percentages were very small, in either case, as compared with those obtained from plates, not of spores alone but also of all organisms capable of forming them.

DATE	TYPE OF SOIL	TEMPERATURE USED	PERCENT MOISTURE IN THE SOIL	NUMBER OF ORGANISMS PER DRY GRAM		
				IN UNHEATED SOIL INFUSION	IN HEATED SOIL INFUSION	PERCENT SPORES
1934	GREENHOUSE					
JUNE	1	75-85°C	17.9	14,616,000	1,045,000	7.2
	2		17.0	19,518,000	735,000	3.8
	3		19.4	25,558,000	674,000	2.6
	4		18.4	18,113,000	598,000	3.3
	5		19.0	26,914,000	1,091,000	4.1
	6			NO COUNTS	NO COUNTS	
AUG	1		17.0	12,771,000	684,000	5.4
	2		17.6	16,578,000	903,000	5.4
	3		16.8	8,654,000	673,000	7.8
	4		17.0	7,229,000	794,000	11.0
	5		17.6	14,563,000	996,000	6.8
	6		17.6	10,012,000	646,000	6.5
1934	FARM					
JUNE	1	75-85 C	10.6	13,855,000	1,323,000	9.7
	2		10.0	11,556,000	1,009,000	8.7
	3		10.5	19,218,000	875,000	4.6
	4		10.6	12,282,000	670,000	5.5
	5		9.5	10,387,000	690,000	6.6
	6		9.0	14,066,000	721,000	5.1
AUG.	1		7.0	12,903,000	649,000	5.0
	2		6.2	8,955,000	582,000	6.5
	3		6.4	7,905,000	455,000	5.8
	4		6.4	7,051,000	440,000	6.2
	5		6.6	10,491,000	563,000	5.4
	6		6.2	10,661,000	606,000	5.7

TABLE 3.—Moisture, organisms, and spores in greenhouse and farm soil in June and August, 1934 as determined at six holes three to five inches apart in a straight line.

A variation of this method was tried on the two soils during June and August, six samples being taken at a depth of three inches from holes three to five inches apart located in a straight line, this in order to determine variations in spore percentages within a very small radius. These samples were numbered 1, 2, 3, 4, 5 and 6. The results are shown in table 3. The counts, both of vegetative organisms and spores, varied quite widely although within the limits of previous counts. The data as to organisms and spores, as determined by the plate counts, can not be deemed to be closely accurate.

Counts of the actinomyces which resisted heating and which were, therefore, presumably in the spore condition are shown in table 4. The

DATE	TYPE OF SOIL	TEMPERATURE USED	PERCENT MOISTURE IN THE SOIL	NUMBER OF ACTINOMYCES PER DRY GRAM		PERCENT SPORES
				IN UNHEATED SOIL INFUSION	IN HEATED SOIL INFUSION	
1933						
FEB.	GREENHOUSE	80-85°C.	30.9	796,000	723	0.09
MAR			18.5	2,319,000	305	0.01
MAY			14.8	940,000	1,000	0.11
JULY			9.1	425,000	1,000	0.24
OCT			20.5	1,257,000	327	0.03
1934						
JUNE		75-85°C.	19.2	1,052,000	2,000	0.2
JULY			8.9	851,000	1,372	0.2
SEPT			17.9	944,000	300	0.03
1935						
FEB			39.6	1,449,000	412	0.03
APR.			19.5	683,000	1,000	0.15
MAY			16.8	1,382,000	400	0.03
JUNE			18.5	755,000	1,000	0.13
JULY			18.8	1,416,000	600	0.04
AUG.			19.0	2,200,000	3,700	0.17
1933						
MAR.	FARM	80-85°C.	11.0	2,102,000	1,700	0.08
JUNE			15.4	650,000	295	0.05
1934						
JUNE		75-85°C.	14.9	3,848,000	2,400	0.07
JULY			4.3	1,724,000	3,000	0.2
AUG.			7.4	1,068,000	2,000	0.2
SEPT.			11.8	1,883,000	1,130	0.06
NOV.			11.0	2,745,000	2,200	0.08
1935						
JAN.			24.5	2,825,000	4,000	0.14
FEB.			28.0	2,813,000	6,500	0.23
MAR			19.0	2,747,000	3,700	0.13
APR.			16.3	1,762,000	3,000	0.17
MAY			19.0	3,550,000	5,000	0.14
JUNE			19.7	3,673,000	10,000	0.27
JULY			14.8	1,886,000	43,000	2.28
AUG			13.0	2,530,000	46,000	1.80

TABLE 4.—Moisture, actinomyces, and actinomyces spores in greenhouse and farm soil, February, 1933 to August, 1935.

very small numbers of actinomyces appearing on the plates from the heated dilutions indicate the scarcity of their spores. As is well understood, these are only dried bits of the mycelium. These plate findings were afterwards confirmed when direct counts of actinomyces on soil smears on a slide were attempted. Nearly all of them seem to occur in the soil in the form of bits of mycelium which are readily killed by temperatures of 75°-85° C., but are more resistant than ordinary vegetative bacteria which die at temperatures between 55°-65° C.

A complete, monthly summary of the years during which the experiment was conducted is shown in the graph on page 15 and also numerically as follows:

TABLE 5.—NUMERICAL SUMMARY BY MONTHS OF THE SEVEN-NINE YEARS COUNTS OF BACTERIA AND ACTINOMYCES IN THE FARM SOIL

Number of months	Month	"Farm soil"			
		Soil moisture	Total number organisms	Number of actinomyces	Actinomyces
		percent	per gram dry soil	per gram dry soil	percent
7	January	26.8	16,929,000	2,537,000	15.
8	February	39.9	19,899,000	3,956,000	20.
8	March	23.1	20,366,000	4,035,000	19.7
8	April	16.5	16,134,000	3,320,000	20.6
7*	May	16.4	17,626,000	3,153,000	17.9
9	June	15.9	17,475,000	3,328,000	19.
8	July	15.2	14,845,000	3,173,000	21.4
7	August	13.2	8,737,000	1,615,000	18.5
8	September	13.9	8,839,000	1,940,000	21.9
7	October	16.4	17,759,000	3,177,000	17.9
7	November	16.1	8,856,000	2,557,000	28.8
8	December	22.2	18,207,000	2,840,000	15.6
	Average				19.6

* Eight months' record on greenhouse soil.

The curves for the farm soil may be somewhat irregular but what might be termed the following tendency manifested by them is much the same but clearer in the greenhouse soil.

1. The soil moisture percentage is highest in the middle of the winter and lowest in the middle of the summer.

TABLE 6.—NUMERICAL SUMMARY BY MONTHS OF THE SEVEN-NINE YEARS COUNTS OF BACTERIA AND ACTINOMYCES IN THE GREENHOUSE SOIL

Number of months	Month	"Greenhouse soil"			
		Soil moisture	Total number organisms	Number of actinomyces	Actinomyces
		percent	per gram dry soil	per gram dry soil	percent
7	January	26.5	21,450,000	2,832,000	13.2
8	February	36.7	26,629,000	5,308,000	19.9
8	March	22.	15,554,000	3,917,000	25.2
8	April	20.3	14,167,000	3,074,000	21.7
7*	May	16.8	12,124,000	2,094,000	17.3
9	June	16.1	12,289,000	2,157,000	17.5
8	July	14.4	10,612,000	2,072,000	19.5
7	August	15.	11,407,000	1,808,000	15.9
8	September	13.9	8,839,000	1,940,000	21.9
7	October	19.	12,282,000	2,640,000	21.5
7	November	20.	17,764,000	3,433,000	18.7
8	December	24.4	14,261,000	2,660,000	19.3
	Average				19.1

* Seven months' record on greenhouse soil.

2. Bacterial counts tend to parallel the soil water content regardless of the air temperature.

3. Actinomyces counts tend to parallel soil water content and total bacterial counts.

The percentage of the organisms in the form of spores varied from 1.3 to 13.9, being between 5 and 10 percent during most of the months.

PLATING-OUT METHOD VS. DIRECT-COUNT METHOD IN SOIL STUDIES OF MICRO-ORGANISMS

The plating-out method ordinarily used has many obvious defects. Only a limited series of species will grow on the nutrient media, some organisms, while present, do not have sufficient vigor to form colonies, and the condition in which they exist in the soil can not be determined. The method whereby bacteria are spread, stained, and counted on glass slides under the oil-immersion lens overcomes some of these objections.

The direct-count method is not described in detail, nor is it essential to review Winogradsky's modification, using erythosin instead of rose bengal followed by a decolorization with acetic acid. All the variations suggested by several authors were tried as were, also, many stains, mordants and times in the hope of securing better results.

The presence and condition of actinomyces in the two soils was the real point of the present study. Their presence, perhaps their numbers, could be determined by making dilutions and plating on agar, but not their condition. For comparative purposes three trials were made in 1932 on the greenhouse soil. A comparison of plate counts and six series of 17 slides each with 50 fields per slide, gave the following results, the first figure representing the plate and the second the slide counts. In order to ensure accuracy in the actinomyces counts, only distinct bits of mycelium were included in the direct counts. I 1,800,000, 1,730,000. II 3,000,000, 2,240,000. III 3,700,000, 3,630,000. Averages 2,833,000, 2,533,000. The two methods gave reasonably close results, the averages of three plate and three direct-counts being 2,833,000, 2,985,000.

CONDITION OF THE SPORES AND ACTINOMYCES IN THE SOIL

The number of bacterial spores has been shown on the graphs for 1933-1935 (pp. 13-15). These data were obtained by heating the soil dilution 1-10,000, which had been used for making the soil plates. The direct-count method was attempted on soil, diluted, stained and examined according to Conn and Thatcher (1927). Soil slides thus prepared did not satisfactorily show the bacterial spores. The vegetative bacteria

were quite readily distinguished, after a little practice, from soil particles and organic débris but the differentiation of spores from coccus or spheroid vegetative species was impossible. However the Winogradsky dye, erythrosin, made possible the differentiation of the spores by staining them a different color.

The usual spore stain, carbol-fuchsin, heated for three to five minutes and then differentiated with acid-alcohol, gave negative results. The spore stain suggested by Schaeffer and Fulton which had worked well with spore material from agar slants was almost as unsuccessful on the soil smears. However it did color certain bodies in the smears in the same manner in which spores are stained, *i.e.*, as green bodies. Whether or not they were spores could not be definitely determined and favorable results were not always obtained. The smears were dried at a high temperature for a long time, as suggested by Lote (1931), but the results were never entirely satisfactory, the soil spores remaining refractory to all the usual spore-staining methods tested.

Apparently the spores in the soil must in some way be changed from those developed in culture tubes, but in just what manner they were altered, whether morphologically, physiologically or chemically, could not be determined. The whole theory of spore development, nature and staining seems involved. This can not be gone into in this paper; however, a good review may be found in Cook (1932). In fact, neither morphology nor colloidal chemistry may have anything whatsoever to do with spores. Their formation, resistance, germination and staining properties may be only the result of the activity or inhibition of certain endoenzymes they contain (Virtanen, 1934).

The actinomyces are undoubtedly of much importance in the soil, especially in respect to changes they induce in the form of its organic matter. Morphologically they seem to exist in the soil in the form of mycelial fragments (plate II, figs. 5, 6), probably attached to bits of decaying organic matter, but in the soil smears they appear as free particles.

These statements concerning the condition of the actinomyces as mycelium are based on much evidence accumulated by the writers. The numbers which appear as colonies on soil plates (table 6) correspond quite nearly to the bits of mycelium—stated numerically—which may be seen on smears by the direct count method. The numbers of actinomyces colonies which appear from soil dilutions which have been used unheated and which give the total numbers of actinomyces of all phases (spore and vegetative) represent mostly vegetative strands since the numbers of actinomyces colonies which appear from the dilutions

which have been heated to a point at which the mycelium is destroyed and only the spores survive, are only a fraction of a percent (table 4). These bits of mycelium are apparently all alive and capable of reproduction.

The writers' determination of the thermal death point of actinomyces mycelium and spores confirms the findings of previous investigators. For some reason, in place of the usual 10 minutes time exposure employed in determining the thermal death point of vegetative bacteria, various times, such as 20 to 30 minutes, have been substituted when actinomyces have been studied. A similar procedure has been followed by the writers who killed the mycelium in liquid cultures in 20 to 30 minutes at 75° C. and the spores in the same time at 85° C.

FIELD EXPERIMENTS IN 1935

The two soils on which bacterial counts had been made during eight years were not chosen at random. They constituted the central portions of the plots used for field experiments on disease resistance completed in 1917. The greenhouse soil had been used only once for this trial and potatoes have been grown on parts of the large plot a few times since 1919. The farm soil, on the other hand, had been tested for the prevalence of scab-producing organisms during three successive years and had not been planted in potatoes since 1916 when the resistance trials were closed. Since 1924 it had been planted to corn for six years, to hay (timothy) for three years, to soy beans (with many weeds) for one year and with a mixture of timothy and clover for one year.

The data on scab resistance on this farm soil derived from both susceptible and varieties were as follows:

Year	Number of varieties	Clean tubers	Sligtly scabby tubers	Badly scabby tubers
		%	%	%
1914	22	27.9	59.9	12.2
1915	21	20.9	52.1	27.
1916	53	5.3	6.9	87.9

No manure was applied, commercial fertilizers only being used, yet the percentage of badly scabby potatoes increased until such susceptible varieties as Green Mountain were almost 100 percent scabbed. Unfortunately, this variety was not planted in 1914, but Gold Coin and Norcross resemble it in skin texture and resistance, so that some idea of the

scab percentage to be expected therefrom can be deduced from their ratios.

Year	Variety	Clean tubers	Sligtly scabby tubers	Badly scabby tubers
		%	%	%
1914	Vermont	11.6	70.8	18.2
	Gold Coin and Norcross	3.1	43.2	43.7
	Average	7.	62.1	30.9
1915	Green Mountain	4.4	42.8	52.8
1916	Green Mountain	...	0.8	99.2

This plot was planted in 1935 with clean formaldehyde-disinfected Green Mountain tubers. No fertilizer or manure was applied since the field had been heavily manured during the preceding two years. An acidity test showed pH 6.8 to 7 as in 1914-1916.

The crop was harvested during the second week of September and every tuber examined for scab and classed as either "clean," "slightly scabby" or "badly scabby." Since these terms may be misunderstood and in order to make the distinctions clear, a series of photographs were taken of typical tubers (plates III and IV). A, B, and C were deemed to be clean tubers but a critical examination of their surface will indicate that such a classification may not be justified. Some rough spots appeared on the A tuber surfaces, much of the B surfaces were shallowly russetted, while part of the C surfaces were shallowly russetted and part more deeply russetted in some regions, probably around lenticels. This russetting, it should be understood, is not as noticeable on the tubers as in the photographs. It is accentuated by a well-known photographic trick, namely, wetting the tubers just before the exposure of the plate or the film. The corky, brown areas absorb water while the white, clean areas do not. In other words, the pictures overstate this condition. Any tuber such as is represented in A, B or C would pass in the market as a clean potato and would be so classed. On the other hand, those pictured under D and E present unmistakable scab lesions, probably the "deep scab" of some investigators. The difference between D and E lies only in the number of the lesions. Attention should be called, however, to the russetted skin located between the real scab lesions.

The data from the harvest was as follows:

Clean tubers	Slightly scabby tubers	Badly scabby tubers	Clean tubers	Slightly scabby tubers	Badly scabby tubers
			%	%	%
5,145	1,945	242	70.2	26.5	3.3

About 50 bushels were harvested from about one-third of an acre. Some regions in the plot showed a high percentage of clean, others developed many scabby tubers. The plot was large enough, however, to enable one to obtain a representative sample.

Since the same divisions into clean, slightly and badly scabbed tubers were made in 1935 as in 1915-1917, and since the senior writer had to do with the sortings both times, the results secured may safely be said to be comparable. The valuable and interesting conclusion can be deduced from the data that, following this long omission of the potato crop from the rotation, either the pathogenic, scab-producing actinomyces died from starvation or else their life habits became so changed that they were no longer capable of infecting the growing tubers at the time when they began to form, producing a lesion of so serious a nature that a deep scab resulted. The russetting which characterized many tubers grown on this plot may perhaps be an initial mild form of scabbing. It is quite conceivable that in the course of years the pathogenic types which produce these mild lesions may again learn how to grow in the cork layer of the tubers and in subsequent years may revert to their bad habits. Possibly several varieties of actinomyces may induce scab. Perhaps russetting is due to the activities of one or more species which may remain almost indefinitely in the soil. Be this as it may, the definite conclusion seems warranted that on some soil types pathogenic strains of actinomyces tend to die out and that a very long rotation which does not include any crop which normally acts as a host will be likely to free such a soil from most of them.

DISCUSSION

The results secured in the long-continued plating out of these soils has led the writer to conclude that the soil water content is the determining factor in respect to bacterial and actinomycelial numbers and that temperature plays only a minor rôle. No antagonism was observed between bacteria (rods and cocci) and actinomyces in the plate counts, the two running reasonably parallel. No explanation is offered touching the correlation between high bacterial count and soil moisture content, but since of all life activities, especially those of growth and division, tend to slow down when low temperatures obtain, one can not help but feel somewhat doubtful as to the validity of the high counts secured on plates representing the winter months.

The question whether actinomyces diminish in numbers and whether the parasitic, potato scab-producing strains tend to die off in the soil when no potatoes were grown on the land for a long time constituted

the gist of the trial. The actinomyces did not increase during the eight years in which counts were made but remained fairly constant in their numbers and in their percentage relation to the total number of organisms. On the other hand, the pathogenic types of actinomyces became changed in some way during this time. The only known test for their continuance is made by planting a susceptible potato variety when, if the scab-producing strains are still present, scabbing results. A test made in this manner showed in 1935 only a small percentage (3.3%) of badly scabby tubers whereas 19 years previously practically all had been scabby. The potatoes counted as clean, however, showed skin russetting either in spots, over large areas, or over the entire tuber. These russetted tubers suggest the possibility that the scab organism, after a long period of deprivation of its host, the potato tuber, is weakened in pathogenicity and produces the true, deep scab only in rare instances. If this hypothesis is correct, the scab-producing actinomyces live saprophytically in the soil when no tubers are available but when the crop is planted again, learn to become pathogenic although only in a mild way, this type of infection leading not to deep scabs but to a russetting of the tuber skin.

General Summary

1. Susceptible varieties of potatoes were grown in 1914-1917 on two plots in order to determine their resistance to potato corky scab. Almost 100 percent badly scabbed tubers were produced in 1916 on one of these soils, a sandy loam, and almost as high a percentage of scab occurred at the same time on the other, a heavy clay loam.

2. The sandy loam soil was not planted to potatoes between 1916 and 1934, timothy, corn, soybeans and oats being grown. A portion of the clay soil plot was planted twice to potatoes during this period.

3. Bacterial counts were made by the plating-out method, 1-1,000,000 dilution, during eight years, beginning in 1924.

4. The total number of organisms, the number of actinomyces, and the soil moisture contents were determined monthly, counts being based on one gram of dry soil.

5. Attempts were made to correlate the total number of organisms to the seasons of the year and to soil moisture contents and to correlate the actinomyces numbers to the total number of organisms, to the season and to the soil moisture contents.

6. The total numbers of organisms were least in the winter months when the soil moisture percentage was highest, the rise beginning in November and the high counts continuing until April.

7. The total number of organisms was lowest in the months May to October, inclusive, when the soil moisture content also was at low ebb.

8. The actinomyces counts roughly paralleled those of the total numbers of organisms, highest in winter and lowest in summer.

9. The numbers of actinomyces neither increased nor diminished during the eight years. They varied somewhat from year to year but were relatively stable.

10. An equilibrium seems to exist between the numbers and relative proportions of the actinomyces and other organisms.

11. The numbers of soil actinomyces as determined on the slides and by plate counts were reasonably concordant.

12. The actinomyces in the soil exist mainly in the form of bits of mycelium, whereas the spores are relatively few in number.

13. In both soils the bacteria were found mainly in the vegetative condition, only from 4 to 10 percent being in the spore stage. The numbers and the percentages of spores were higher in the sandy loam than in the clay loam. The spore numbers and percentages followed closely the total number of organisms month by month.

14. The spores of the soil bacteria did not color with any of the differential spore stains although they were physiologically spores in their resistance to temperatures ranging from 75° to 80° C.

15. Disinfected Green Mountain potato seed was planted on the sandy loam soil, no potatoes having been grown thereon in 19 years. The crop carried a high percentage of clean tubers. The 1916 yields were: 0.0 percent clean; 0.8 percent slightly scabby; 99.2 percent badly scabby; the 1935 yields 70.2 percent clean, 26.5 percent slightly scabby, and 3.3 percent badly scabby.

16. A russet skin on a normally white-skinned tuber may be the result of an actinomyces infection. Although very superficial the russetting is often localized in such a manner that it implies lenticel infection.

17. The loss from once infected soil of pathogenic species of actinomyces which will produce typical deep scab might be explained as follows:

a. The pathogenic species or strains may have died out in the soil from lack of a host suitable for pathogenic growth.

b. Many species or strains may have pathogenic tendencies but, in case no host is present, may lose them. They may regain these pathogenic habits if provided with a suitable host. The first indications of a

return of the disease is its reappearance in a milder form; in the case of potato scab this would be russetting.

The senior writer is inclined to believe that the second suggestion is more likely to represent the truth; however further work is needed before the last word is said on this subject.

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